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An active pathway for serotonin synthesis by renal proximal tubules

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An active pathway for serotonin synthesis by renal proximal tubules. Serotonin (5HT) has significant effects on renal metabolism and glomerular function and is a potent renal vasoconstrictor. In this study we describe and localize a highly active biosynthetic pathway for serotonin in the kidney. Rat kidneys were dissected into cortical and medullary fractions; in some experiments the cortex was also separated into subfractions enriched with glomeruli or proximal tubules. Serotonin and tryptophan hydroxylase (TyOH) were measured by radioenzymatic techniques.

	Medulla	Cortex	Glomeruli	Proximal tubules	Brainstem
5HT, *ng/g*	32.39 ±1.49	7.13 ±0.77*	—	—	510.7 ±10.0
TyOH, *nM/30min/g*	0	19.33 ±0.77*	0.83 ±0.78	31.33 ±3.23*	11.88 ±0.37

* $P < 0.05$ cortex vs. medulla; tubules vs. glomeruli

Renal denervation did not alter tryptophan hydroxylase activity. In kidneys from human cadaveric donors, cortical tryptophan hydroxylase (4.13 ± 0.68 nM/30 min/g) exceeded that in the medulla (1.96 ± 0.86 nM/30 min/g). Aromatic L-amino acid decarboxylase, the remaining enzyme for serotonin synthesis, is present in both rat renal cortex and medulla; however, we found 15–fold greater decarboxylase activity in proximal tubular (2070 nM/30 min/g) as compared to glomerular (131 nM/30 min/g) subfractions. We were able to demonstrate that under physiological conditions, free urine serotonin reflects actual biosynthesis by the kidney. Thus, although serotonin stores retained by the kidney appear small and relatively localized to the medulla, the enzymatic activity for the synthesis of serotonin in the kidney is comparable to that in the brain, with the complete pathway localized to renal cortical proximal tubules. These data suggest that further studies of renal serotonin metabolism may contribute to our understanding of renal function in health and disease.

Serotonin is a well established brain neurotransmitter. Rich stores of serotonin also exist in some tissues outside of the central nervous system; actual serotonergic nerves have been identified in the enteric nervous system of mammalian gut [1] and in the nodose ganglia [2]. The peripheral administration of serotonin or its precursors results in a variety of physiologic effects, and serotonergic receptors which mediate many of these responses have been identified. In particular, the administration of serotonin has been shown to have significant effects on renal metabolism, glomerular function and renal vascular tone [3, 4]. Furthermore, recently developed serotonin-2 receptor antagonists, such as ketanserin, appear to ameliorate some forms of hypertension and possibly congestive heart failure [5, 6]. These studies have rekindled an interest in the possible role of serotonin in the pathophysiology of cardiovascular and renal disease.

Serotonin is synthesized from its essential amino acid precursor tryptophan. The first step in the conversion is catalyzed by tryptophan hydroxylase, the rate-limiting enzyme. The resultant product, 5-hydroxytryptophan, is subsequently decarboxylated by aromatic amino acid decarboxylase to serotonin (5-hydroxytryptamine). Serotonin is degraded primarily by monoamine oxidase and the aldehyde formed is rapidly oxidized to 5-hydroxyindoleacetic acid (5-HIAA).

The kidney, particularly the renal tubular cells of the cortex, is known to be an extremely rich source of aromatic amino acid decarboxylase [3, 7, 8, 9]. Substantial monoamine oxidase activity, also residing almost exclusively in the renal cortex and in renal tubular cells, has been described as well [3, 10]. However, to our knowledge, no quantitative assessment or localization of renal tryptophan hydroxylase has been published, although its presence in the kidney was qualitatively identified by Cooper and Melcer [11]. In this study we examine in more detail the possibility that an active pathway for serotonin biosynthesis exists in the kidney.

Methods

Preparation of tissues

General. Male Wistar rats, approximately 12 weeks of age, were used in the experiments characterizing renal serotonin and its synthesis. A 12 hr light, 12 hr dark cycle was maintained in the animal housing area. The rats were allowed water, but were deprived of food for approximately 20 hours prior to the experiments which were performed from 1000 to 1400 hours. The rats were anesthetized with pentobarbital (60 mg/kg i.p.) and heparinized (8,000 U USP/kg). The kidneys were exposed and flushed free of blood in situ with 10 ml heparinized saline administered through a hypodermic syringe into a cross clamped abdominal aorta. The kidneys were removed, cleaned,

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Table 1. Serotonin metabolism in the kidney and brainstem of the rat

	Medulla[a]	Cortex[a]	Glomeruli[b]	Proximal tubules[b]	Brainstem[b]
Serotonin, *ng/g*	21.4 ± 1.5	7.1 ± 0.8*	—	—	510.7 ± 10.8
Tryptophan Hydroxylase, *nM/30 min/g*	nd	19.3 ± 0.8*	0.08 ± 0.8	31.3 ± 3.2*	11.9 ± 0.4

Abbreviations: nd, not detectable.
* $P < 0.001$ proximal tubules vs. glomeruli or cortex vs. medulla.
[a] $N = 8$ for medulla and cortex.
[b] $N = 6$ for nephron fractions and brainstem.

dissected into medulla (including papilla) and cortex on an iced plate, weighed and then frozen on dry ice. In some experiments brainstem, consisting of midbrain, pons and medulla, was also taken for assay. Human kidneys, from cadaveric renal donors which could not be used for transplant, were also examined. These kidneys were perfused with blood-free media for approximately 24 hr prior to dissection and assay.

Subfractionation. Proximal tubular fragments were separated from glomeruli and distal tubular fragments by a modification [7] of the procedure described by Vinay et al [12]. The method [7] involves collagenase digestion of kidney cortex slices followed by density gradient centrifugation through Percoll. Four distinct layers were obtained; these were recentrifuged, and the pellets were allowed to dry for two minutes before weighing to obtain wet weight. Expressing the results in terms of protein content had no effect on the value of each fraction relative to the other. The composition of each fraction was checked by light microscopy, staining for alkaline phosphatase activity and gluconeogenic activity. Data for layer one (60 to 80% glomeruli) and layer four (85 to 95% proximal tubules) are listed in Table 1. Layers two and three which exhibited a mixture of tissue fractions had intermediate values.

Denervation. Kidneys were surgically denervated by stripping the renal artery and coating it twice for ten minutes each time with cotton soaked in 10% phenol in alcohol approximately seven days before assay. Denervation using the neurotoxin 5,7-dihydroxytryptamine, (5,7 dihydroxytryptamine creatinine sulfate, Regis Chemical Company, Morton Grove, Illinois, USA) was performed exactly as described by Sole et al [13].

Perfusion. Kidneys from 250 to 350 g male Wistar rats were prepared for isolated perfusion as previously described [14]. They were perfused with a modified Krebs-Henseleit solution containing 6.7% dialysed bovine serum albumin (Fraction V, Miles Laboratory, Toronto, Ontario, Canada), 5 mM glucose and a mixture of 18 amino acids (12.5 mM) containing 0.08 mM tryptophan. The perfusate was gassed in a water-jacketed film oxygenator with 95% oxygen/5% carbon dioxide. The perfusion pH was 7.42 and the temperature was 37.5 to 38°C. Perfusions were carried out with a renal artery pressure of either 90 mm Hg or 110 mm Hg. Urine was collected every 10 min with perfusate samples taken at the mid point. Tritiated inulin (New England Nuclear, Boston, Massachusetts, USA) was added to the perfusate for measurement of inulin clearance. Sodium was measured by flame photometry and inulin radioactivity was counted on scintillation counter after adding samples to 11.5 ml ACS aqueous counting fluid (Amersham Corp., Oakville, Ontario, Canada) with a total of 1.5 ml water.

Biochemical Assays

Tryptophan hydroxylase. Tryptophan hydroxylase activity was measured by appropriate modifications to the method of Waymire et al [15] for tyrosine hydroxylase. In this sensitive assay, the substrate, carboxyl-labelled tryptophan, is converted by tryptophan hydroxylase to carboxyl-labelled 5-hydroxytryptophan. The latter amino acid is preferentially decarboxylated after the addition of an excess amount of aromatic amino acid decarboxylase, and the radioactive CO_2 liberated in the process is collected and measured. The tissues to be assayed were harvested as described above; they were then homogenized with a Polytron homogenizer (Brinkman Industries, Toronto, Ontario, Canada) in approximately 11 volumes (wt/vol) of ice cold 0.1 M potassium phosphate buffer, pH 7.4, containing 0.1% Triton X 100. The homogenates were gently stirred for twenty minutes at 4°C and then centrifuged for ten minutes at 10,000 *g*; the supernatant was taken for assay. The assay medium contained (in a total volume of 320 μl): 220 μl tissue supernatant, 70 μl reaction mixture and 30 μl $^{14}CO_2$-tryptophan (53.2 mCi/mM). The reaction mixture was made as follows: 1.275 mg pyridoxal phosphate and 9.23 mg dl-6-methyl-5, 6, 7, 8-tetrahydropterine were dissolved in 1.5 ml, 0.01 M sodium phosphate pH 7.0; this solution was Medium 1. Medium 2 consisted of 1.5 ml, 1 M potassium phosphate pH 6.2, 40 μl mercaptoethanol, 0.24 ml catalase 1:4 with 0.01 M sodium phosphate pH 7, plus water to a total volume of 5.0 ml. Medium 1 (0.75 ml) Medium 2 (2.50 ml) and aromatic amino acid decarboxylase (1.0 ml) were combined immediately before the assay to constitute the reaction mixture. Blanks were prepared by boiling the tissue supernatant. The remainder of the tryptophan hydroxylase procedure (i.e., incubation, termination, collection of $^{14}CO_2$ and counting) was exactly as described by Waymire et al [15] for tyrosine hydroxylase. The assay was linear for enzyme content over the 30 min incubation. $^{14}CO_2$ collection was complete (100%) two hours following termination of the reaction by trichloroacetic acid. Renal cortical specimens gave values which were approximately 20 times blank.

Aromatic amino acid decarboxylase was prepared from liver tissue taken from rats pretreated with p-chlorophenylalanine, 100 mg/kg for three successive days, to irreversibly inhibit any tryptophan hydroxylase activity [16]; the rest of the preparative procedure was exactly as described by Kizer et al [17]. We were unable to detect any residual tryptophan hydroxylase activity in such preparations.

Miscellaneous assays. Serotonin was measured by the radioenzymatic method of Hussain and Sole [18]. Tryptophan was measured by the method of Bloxam and Warren [19]. Blood and

Table 2. Effect of denervation on rat renal cortical tryptophan hydroxylase activity (nM/30 min/g)

	Study 1[a]	Study 2[b]
Control	20.4 ± 1.6	27.3 ± 0.5
Surgical denervation	21.0 ± 2.1	—
5,7-dihydroxytryptamine	—	25.6 ± 2.8

[a] $N = 8$.
[b] $N = 3$.

platelet poor plasma was prepared and assayed exactly as described by Hussain and Sole [18]. Catecholamines were measured by the method of Sole and Hussain [20], modified as described in Sole et al [21]. Urine cyclic AMP was assayed by the method of Frandsen and Krishna [22]. Antisera to cyclic AMP was purchased from Miles-Yeda Ltd. (Elkhart, Indiana, USA).

Results are expressed as mean ± SEM. Statistical significance was calculated using Student's two-tailed t-test (unpaired).

Results

Relatively little serotonin could be found in the kidney; "stores" in the medulla exceeded those in the cortex approximately threefold (Table 1). On the other hand, relatively large levels of tryptophan hydroxylase, the rate-limiting enzyme for serotonin production, were found. This enzyme was localized by differential density gradient centrifugation to the proximal tubular subfraction of the renal cortex where its activity exceeded that in the brainstem (Table 1). We could not detect tryptophan hydroxylase activity in heart, aorta, or adrenal gland, in spite of significant serotonin stores in these organs. Aromatic amino acid decarboxylase activity was measured and was also found to be concentrated in the proximal tubular subfraction (2070 nM/30 min/g proximal tubular vs. 131 nM/30 min/g glomerular subfraction).

Renal cortical tryptophan hydroxylase did not appear to be localized in serotonergic nerve terminals for neither surgical denervation nor treatment with the neurotoxin 5,7-dihydroxytryptamine had any effect on enzyme levels (Table 2). As 5,7-dihydroxytryptamine is neurotoxic to both noradrenergic and serotonergic nerve endings, the effectiveness of the administered regimen was confirmed by a decrease in renal cortical norepinephrine from 102.3 ± 7.2 ng/g to 26.1 ± 5.7 ng/g. Surgical denervation reduced renal norepinephrine from 109.3 ± 9.7 ng/g to 4.85 ± 0.69 ng/g.

The ability of the kidney to synthesize serotonin from tryptophan was confirmed in a series of experiments in the isolated rat kidney perfused with a solution (free of serotonin and other amines or 5-hydroxytryptophan) containing 0.08 mM tryptophan, other amino acids and 5 mM glucose at 90 mm Hg. At 35 minutes we found 0.51 ± .28 ng/ml of serotonin had accumulated in the recycled perfusate (70 ml). Serotonin production in urine collected between 30 and 40 minutes was 0.65 ± .10 ng/min/g. Renal perfusion at 110 mm Hg, although increasing GFR and sodium excretion, did not affect serotonin production.

Mammalian urine is known to contain significant quantities of serotonin. In order to estimate the contribution of renal production, as opposed to that filtered at the glomerulus, we administered p-chlorophenylalanine (PCPA), a specific inhibitor of tryptophan hydroxylase to rats. PCPA is known to have no effect on either monoamine oxidase or aromatic amino acid decarboxylase [16]. Administration of PCPA led to a marked and proportionate fall in both renal cortical tryptophan hydroxylase activity and urine serotonin excretion. Blood serotonin exhibited no change (Table 3).

Table 3. Effect of inhibition of tryptophan hydroxylase on urine serotonin (5HT) in rats

	Renal cortical tryptophan hydroxylase *nM/30 min/g*	Urine 5HT *μg/24 hr*	Blood 5HT *μg/ml*
Control[a]	19.5 ± 2.0	2.13 ± 0.17	2.87 ± 0.62
PCPA[b,c]	4.4 ± 0.3*	0.65 ± 0.09	2.34 ± 0.21

[a] $N = 8$.
[b] $N = 10$.
[c] *P*-chlorophenylalanine (316 mg/kg of the methyl ester ip) was given to rats between 0900 and 1000 hr. Urine collection began four hr later and continued for 24 hr at which time blood and kidneys were taken for analysis.
* $P < 0.001$, PCPA vs. control.

Table 4. Tryptophan hydroxylase activity (nM/30 min/g) in human kidney

Patient	Medulla	Inner cortex	Outer cortex	Small arteries[a]
1	2.52	3.14	4.49	nd
2	0.28	4.15	5.89	nd
3	3.10	4.85	5.40	nd

Abbreviations: nd, not detectable
[a] hand dissectable, intrarenal arteries (0.5 mm-2.0 mm diameter)

In our next series of experiments we wished to ascertain whether these results were relevant to man. We obtained three human kidneys from cadaveric renal donors which ultimately could not be used for renal transplant. These kidneys were not affected by disease and had been isolated and perfused for approximately 24 hr before release for experimentation. Significant quantities of tryptophan hydroxylase were found in human kidneys, but the distribution of the enzyme was more uniformly divided across the kidney than in the rat. Small, but hand-dissectable intra-renal arteries exhibited no enzyme activity (Table 4).

We could not administer PCPA to inhibit renal tryptophan hydroxylase and examine urine serotonin in man. On the other hand, if renal tryptophan hydroxylase is relatively unsaturated with its substrate, tryptophan, serotonin synthesis may be stimulated by the administration of this precursor amino acid [23]. We provided a dietary supplement of tryptophan to four normal volunteers. Blood and platelet poor plasma serotonin did not change after tryptophan administration; however, urine serotonin reflected plasma tryptophan showing a more than fivefold increase (Table 5). Urine cyclic AMP remained within the normal range and did not appear to exhibit a major change (Table 5).

Table 5. Effect of dietary tryptophan supplements on urine serotonin (5HT) and cAMP in normal man[a]

Volunteer	Plasma tryptophan *μg/ml*	Blood 5HT *ng/ml*	Urine 5HT *nM/hr*	Urine 5HT × 10^6 creatinine	Urine cAMP *nM/hr*	Urine cAMP × 10^4 creatinine
Control						
1	1.98	156	29.2	63.5	66.6	1.47
2	1.01	86	32.0	57.5	—	—
3	1.01	153	18.7	38.2	—	—
4	0.96	140	23.9	39.8	70.3	1.17
Tryptophan						
1	7.31	198	190.1	265.7	98.6	1.38
2	3.81	107	147.6	290.1	81.7	1.61
3	8.06	133	145.9	299.1	65.3	1.34
4	6.64	153	99.9	157.6	82.4	1.30

[a] 1.5 g of L-tryptophan free base (Sigma Chemical Co., St. Louis, MO) was administered in capsule form with breakfast at 0700 and 0800 hours [34]. Urine was collected for the next six hours. Blood was taken for analysis at three hours.

Discussion

In this study we show that renal tryptophan hydroxylase activity in the rat is equivalent to, or greater than, that found in the brain stem. Furthermore, this activity appears localized to the renal cortex, specifically in a subfraction highly enriched in proximal tubules. Surgical denervation or the administration of a serotonergic neurotoxin failed to alter enzymatic activity; thus, it appeared that renal tryptophan hydroxylase was not dependent on an extrinsic renal innervation or the presence of serotonergic nerve terminals.

Although we measured this enzymatic activity in kidney homogenates, our data appears to meaningfully reflect the capacity of the whole kidney to synthesize serotonin from its precursor amino acid tryptophan. We measured the accumulation of significant quantities of serotonin in both the perfusate and the urine of isolated kidneys perfused by tryptophan-containing, but an initially 5-hydroxytryptophan and serotonin-free solution.

Substantive quantities (100 to 500 ng/g) of serotonin in the kidney have been reported by earlier workers. However, these studies, performed several years ago [24, 25], were incapable of distinguishing serotonin from related indoles (or many other interfering substances), and the methods of tissue preparation suffered from significant contamination by platelets or mast cells with their accompanying serotonin stores. Using a highly specific assay for serotonin combined with a careful flushing of the kidney and stripping of the renal capsule to minimize such contamination, we found renal serotonin stores to be relatively small. Whether the threefold increase in medullary, as compared to cortical, serotonin represents an actual heterogeneity in distribution or a flushing artefact is uncertain.

Serotonin and its major metabolite 5-HIAA appear in significant quantities in mammalian (including human) urine [3, 4, 24, 26]. Sulphate and glucuronide conjugates are also present [3, 4, 26]. The major excretory product is 5-HIAA which is both filtered at the glomerulus and secreted actively by the renal tubules [27]. Almost all urinary 5-HIAA is said to come from the abundant serotonin stores of the gastrointestinal tract [28]. Serotonin presented to the kidney is secreted by the ordinary organic base transport system [3, 27, 29]. However, systemic (or parenteral) serotonin administration has virtually no effect on the levels of free serotonin in the urine, but instead markedly influences the excretion of 5-HIAA and serotonin conjugates [30]. Furthermore, in carcinoid syndrome, metabolites of serotonin such as 5-HIAA increase multifold more times in the urine than does the indolealkylamine itself [26, 31]. These data appear to reflect the fact that serotonin presented to the bloodstream is either rapidly sequestered in platelets, or is efficiently metabolized in either the hepatic or pulmonary circulations, leaving very little available for filtration at the renal glomerulus (this may not be the case if serotonin metabolism or uptake is inhibited by drugs or disease). Thus, under physiological conditions at least, it is reasonable to suggest that urine serotonin may reflect actual biosynthesis by the kidney. Our experiments support this hypothesis, for we observed a marked decrease in serotonin excretion in the urine in the absence of a significant change in blood serotonin levels, following the inhibition of tryptophan hydroxylase by PCPA (Table 3).

These data also appear to be relevant to man. We found significant tryptophan hydroxylase activity in the human kidney; there was less cortical but more medullary activity than that seen in the rat. In order to determine the renal contribution to serotonin excretion in human urine, we took advantage of the fact that under most physiological circumstances, tryptophan hydroxylase is unsaturated relative to its substrate tryptophan, and thus the availability of this essential amino acid to the enzyme can be a major determinant of the control of serotonin synthesis [23]. Tryptophan filtered at the glomerulus appears to be reabsorbed specifically in the renal proximal tubules [32, 33]—the putative major site for renal serotonin synthesis. We, therefore, administered to human volunteers a dietary supplement of tryptophan which increased plasma tryptophan severalfold; this regimen had no significant effect on blood or platelet poor plasma serotonin [34, Table 5]. We observed an approximately fivefold increase in urine serotonin over a period of six hours following the supplement. Thus, urine serotonin in man also reflects renal synthesis under physiological conditions. Such a relationship would be expected to facilitate future studies determining the role of renal serotonin production in health and disease.

The pathophysiological significance of renal serotonin production is speculative. The renal vasoconstrictive effects (particularly in the renal cortex) of exogenously administered serotonin are well known [3, 4, 35]. Renal perfusion with 5-hydroxytryptophan (the product of tryptophan hydroxylation

and the immediate precursor of serotonin), has been reported to increase renal vascular resistance; this response can be blocked by decarboxylase inhibition or serotonin receptor antagonism [36].

These data suggest a role for peripheral, perhaps renal, serotonin as a modulator of renal vascular resistance. We found no change in serotonin production or excretion, however, in our isolated perfused kidney experiments when the perfusion pressure was increased from 90 to 110 mmHg. Furthermore, no measurable tryptophan hydroxylase could be found in the small intrarenal arteries of human kidney.

Serotonin is a potent stimulator of renal cortical (particularly glomerular) cyclic AMP [4, 37] and thus renal biosynthesis of serotonin may be a physiological regulator of glomerular function, or structure, or play an important role in the pathogenesis of glomerular disease. Such considerations are particularly important in light of recent experimental evidence which suggests that manipulation of dietary protein can ameliorate or stop the progression of chronic renal disease in certain animal models [38]. We tested these hypotheses only superficially. We failed to find evidence for tryptophan hydroxylase in the renal glomerular subfraction. Furthermore, urine cyclic AMP remained within normal limits in spite of a fivefold increase in urine serotonin excretion after tryptophan feeding (Table 5).

The difficulty in ascribing a biologically significant role for renal serotonin production exhibits many parallels to similar problems encountered in studies of renal dopamine. In the kidney, both compounds have a rapid turnover, low storage and can be produced outside of monoaminergic nerve terminals (particularly in the proximal tubules) [14]. Both may influence presynaptic adrenergic receptors and norepinephrine release [39, 40, 41]. Also, there is evidence to suggest that both of these amines have a direct effect on electrolyte transport by tubular epithelium [3, 42, 43]—but in both cases the evidence is highly controversial. Both of these amines are also produced by the carotid body and sensory ganglia, where they may act as modulators of afferent or reflex nerve traffic [44, 45]. In many cases these two monoamines appear to have opposing biological effects; for example, they have apparently opposite effects on renal hemodynamics and function [3, 35, 46, 47]. Perhaps both serotonin and dopamine ultimately function as significant components of a tubular-glomerular feedback system and a means of renal auto-regulation.

Finally, one other consideration arises from our studies. Some assays for tryptophan hydroxylase employ crude preparations of aromatic amino acid decarboxylase as part of their methodology [48]. The kidney is a particularly rich and often-used source of this enzyme. It is evident from the data we have presented that these semi-purified preparations of kidney decarboxylase probably contain significant quantities of contaminant tryptophan hydroxylase—introducing a potentially important source of assay artefact. Enzyme derived from PCPA-treated rat liver as described in this study [17] exhibits no intrinsic tryptophan hydroxylase activity and thus should be used in such analyses.

In conclusion, we have shown that the kidney has a considerable extraneural capacity to synthesize serotonin from circulating tryptophan. It appears that the proximal tubules, the site of tryptophan reabsorption, are most active in this biosynthesis. Free serotonin in the urine appears to reflect renal production under physiological conditions. This non-invasive reflection of synthesis should facilitate future investigations pertaining to the biological significance of these observations both in animals and in man.

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